Plants Need Vitamins Too*

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Thirty years ago when I first became interested in the nutrition of the fungi the failure of a fungus to grow or grow well in a medium of known composition was ascribed to a variety of causes, none accounting satisfactorily for the results. Mycologists recognized that many fungi required special media containing some material of natural origin; and oatmeal, corn meal, potatoes, bean pods, extract of malt, peptone, wood, dung and many other natural products were frequently used as such or incorporated in the material upon which these organisms were grown. Generally speaking, an effort was made to supply as food the material on which the organism grew in nature.

The advantage of such natural media was not understood. Some suggested that it was because of the suitability of the minerals in the natural product, or its favorable acidity or alkalinity, to the presence of a particular carbohydrate or some unique source of organic nitrogen, to the special water relations afforded by the material or to some physical property. We know now that the growth of many fungi is conditioned by the presence in the medium of minute traces of specific organic compounds, some of them identical with the known vitamins; and the presence of these growth substances in products of natural origin frequently accounts for their advantages as culture media. This was a possibility seriously considered by few, if any, of those concerned with the cultivation of fungi thirty years ago. In fact, the very word vitamin was unknown at that time; it was coined by Casimir Funk in 1912 and up to eight years ago not a single completely convincing example of the importance of a vitamin for a plant could be cited.

During the period from 1912-1934 the animal physiologist proceeded to demonstrate the importance of vitamins for the growth and well-being of animals and to explore their multiplicity, functions, sources and chemistry. It was generally agreed that plants were the sources from which animals in the last analysis obtained their vitamins, or in other words, that plants made vitamins and animals used them, a fortunate circumstance for us and a sort of philanthropic activity on the part of plants. But the possibility that vitamins were important in the metabolism of the plant itself was regarded by the majority of plant physiologists with concealed or open scepticism. In fact, a complete and satisfactory demonstration of the importance of vitamins for plants waited, as so frequently happens in science, on advances in another field, on the isolation of a vitamin in chemically pure form. Crystalline thiamine (vita-

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* Delivered at the 75th Anniversary Celebration of the Torrey Botanical Club at the American Museum of Natural History, Wednesday evening, June 24, 1942.

min B_1) was isolated by Jansen and Donath in 1926 and became generally available in 1934. In that year Schopfer showed that the bread mold *Phycomyces* would not grow unless it was furnished with minute traces of this vitamin. With this convincing demonstration as a basis and the isolation of additional vitamins in chemically pure form our knowledge advanced rapidly.

Now we realize that plants are not so philanthropic as they once seemed. We know they too need vitamins, but more provident than animals, most plants make their own vitamins. Only the minority, and these chiefly the lower plants, suffer from vitamin deficiencies; that is, they cannot develop unless the material upon which they grow contains some of the necessary vitamins which, of course, must come from some other kind of plant or from an animal which has obtained them from a plant. Some bacteria, yeasts and molds need to be supplied with vitamins. Few, if any, of the trees, vegetables, flowers and other green plants benefit from having vitamins supplied them. To the best of our knowledge they make all they need.

You may ask whether this means, in spite of the considerable publicity on this subject, that supplying green plants with vitamin B_1 or other vitamins is not beneficial? I would answer this question in this way. The application of vitamins to trees, flowers, vegetables and other green plants is still in an experimental stage. Some investigators have reported beneficial results on some kinds of plants and not on others. Many have obtained negative results. We must conclude either that the conditions under which vitamins are beneficial to green plants are poorly understood or that their application does not bring favorable results. Certainly the use of vitamins in horticultural practice does not accomplish the miracles some would have us believe, and no reputable horticulturist on the basis of the evidence now at hand would recommend their use under normal garden and greenhouse practice.

In discussing the relation of vitamins to plans there are a good many questions we might ask. For example, what is a vitamin, how were they discovered, how do we know plants need vitamins and how many vitamins do plants need, what plants must be supplied vitamins and what do the vitamins do in the plant, how much of a vitamin is needed and is there a substitute for a particular vitamin—something just as good? I can't answer all these questions for any one vitamin, but some of them can be answered by discussing a particular vitamin, and I have selected three, thiamine or vitamin B_1, pyridoxine or vitamin B_6 and biotin or vitamin H.

Thiamine. Thiamine or vitamin B_1 is a white crystalline substance containing carbon, hydrogen, nitrogen, oxygen and sulfur. Its empirical formula is $C_{12}H_{16}ON_4S$. Its structure is known and between 25 and 30 tons are now made annually in chemical laboratories in this country. In 1935 thiamine cost $300 per gram which is at the rate of $135,000 per pound. With the discovery of methods of making it synthetically and the development of mass production its price dropped to $.53 per gram or about $238 per pound. Thiamine is used in the treatment and prevention of beri beri, of lack of appetite in children and of various types of neuritis and in the enrichment of flour. It is perhaps the best known and most widely advertised of all the vitamins.

The history of our acquaintance with this vitamin begins with attempts to cure a disease common in the far east, recognized by the Chinese as early as 2697 B.C. and known as beri beri. In the 19th Century it was found that beri beri could be cured by controlling the diet; for example, Takaki, Surgeon General of the Japanese Navy, about 1885, substituted meat and legumes for part of the rice in the diet of sailors and reduced the incidence of beri beri from between 30 and 40 percent to less than ½ percent. Takaki believed that this was because of the increased protein furnished. In 1912 Casimir Funk, a Polish scientist, suggested that beri beri was the result of the lack of a specific organic substance in the food. This new dietary essential he called a vitamin. It was found that pigeons and other animals fed on polished rice developed a type of beri beri which could be cured by feeding rice polishings or extracts made for them. The next step in logic was to assume that if vitamins were really present in rice polishings and not merely in the minds of Funk and those who believed as he did vitamins could be isolated and their chemical nature determined. Many made the attempt. Two Dutch investigators in Java, Jansen and Donath, succeeded in isolating a small quantity of vitamin B_1 in 1926, but its isolation in quantity and its synthesis in the laboratory were not accomplished until 1934. Since 1934 many yeasts, bacteria, filamentous fungi, and the excised roots of a number of higher plants have been found to suffer from thiamine deficiencies. It has been found further that those plants which grow without an external supply of thiamine make it, and the conclusion has been reached that all living organisms need thiamine. Some make it from simpler substances, others must be supplied with it. It is as essential and as necessary as water or minerals or any other indispensable item in the nutrition of an organism. Its absence means death, its presence, life.

Pyridoxine. Pyridoxine or vitamin B_6 is also a white crystalline compound. Its empirical formula is $C_8H_{11}O_3N$. It is a derivative of an ill-smelling liquid known as pyridine. In 1939 pyridoxine cost $12.00 per gram. It can be purchased now for $3.00 per gram or about $1350 per pound. The medical value of pyridoxine is ill-defined. It may be of value in the treatment of certain muscular rigidities, of paralysis agitans and perhaps of other conditions.

In 1915 Goldberger, a United States Public Health Official, recognized that dietary deficiencies might play an important part in the development of pellagra, a condition affecting between 400,000 and 500,000 people annually. In 1937 Elvehjem at the University of Wisconsin demonstrated that nicotinic acid would cure a pellagra-like condition in the dog known as "black tongue" and in 1938 Spies and coworkers reported that nicotinic acid was effective in the treatment of human pellagra. During the course of Goldberger's work he was able to produce a syndrome in rats which he called "rat pellagra." However, György (1934 and 1935) at the Babies and Children's Hospital in Cleveland determined that this condition was not cured by the pellagra-preventing factor, by thiamine or by vitamin B_2. It could be cured by particular extracts of rice polishings, and he proposed that the deficiency in the food causing this peculiar type of dermatitis was a new vitamin which he called vitamin B_6. In 1938 vitamin B_6, later named pyridoxine, was isolated and identified by Kuhn in Germany, Ichiba and Michi in Japan, Lepovsky in California, György in Ohio and Keresztesy and Stevens in New Jersey. It was synthesized in 1939 by Harris and Folkers. Partial or complete deficiencies for pyridoxine have been found for some bacteria, some yeasts and a good many fungi. It too appears to be a vitamin needed by all living organisms.

Biotin. Biotin is a white crystalline substance which in the form of its methyl ester has the empirical formula $C_{11}H_{18}N_2O_3S$. Its structural formula is not yet known, and it has not been synthesized from simpler substances. It may be obtained by a long and costly process of purification from natural products such as egg yolk or liver and for $10.00 you may purchase 75 micrograms of pure biotin which is at the rate of about $62,400,000 per pound. It was first isolated by Kögl and Tönnis of Utrecht in 1936 from the yolk of eggs and has proved to be the most potent of all the vitamins. Kögl and Fries were able to detect the effect on the growth of a fungus of 0.0001 of a microgram of biotin methyl ester. Biotin is widely distributed in products of natural origin. We have found it in such unexpected places as cow manure and cotton. In fact, a bale of cotton contains about $1000 worth of biotin. It is made by green plants and many bacteria, yeasts and filamentous fungi. There are, however, a good many of the lower organisms which lack the ability to make biotin; some cog is missing in their machinery, or it works slowly, and these organisms grow poorly or not at all in media from which this vitamin is absent. It is probably essential for animal growth and from recent pronouncements in various journals may be intimately associated with the development of cancer.

The discovery of biotin has a long and interesting history. In 1860 Pasteur published an important memoir on alcoholic fermentation in which he came to the conclusion that yeast grew if supplied with yeast ash, ammonium salts and a fermentable sugar. He observed that the fermentative power of yeast was increased by the addition of extracts from natural products, for example, grape juice, sugar beet juice or yeast juice but all the essentials for growth were included, according to Pasteur, in a solution of yeast ash, ammonium salts and glucose. However, in 1869 the famous German chemist, Justus von Liebig, stated that yeast neither grew nor fermented sugar under the conditions defined by Pasteur. This criticism was so keenly felt that in 1872 Pasteur declared he was so sure of his results that he was prepared to perform the experiment in the presence of Liebig himself. The demonstration never took place, Liebig died in 1873, and the nutritional requirements of yeast as defined by Pasteur remained unchallenged for many years.

In 1901 Wildiers of Belgium reported that yeast would not grow under the conditions defined by Pasteur if the amount of yeast used in the seeding was small. He found that small amounts of a thermostable organic material were necessary for the growth of yeast and gave to this chemically undefined material the name, bios. Bios was a concentrate prepared from the yeast itself. Wildiers suggested that Pasteur obtained his results because he had used a large quantity of yeast for the seeding, and this large seeding had carried with it sufficient bios to permit growth.

Wildiers' proposal that minute traces of organic material in addition to minerals, ammonium salts and sugar were necessary for yeast growth was roughly handled by some of his contemporaries, including Fernbach (1902) Windisch (1902) and Pringsheim (1906). Various students in Wildiers's laboratory supported his proposal but since no one could identify bios chemically, it remained for 20 years before the bar of science with the verdict, proposed but unproven.

In 1921 MacDonald and McCollum reported that yeast would grow in a solution of cane sugar and inorganic salts, but 2 years later Funk and Friedman demonstrated that ordinary cane sugar may contain a growth activator of organic character which required for its removal three crystallizations of the sugar from alcohol. And so after 60 years the dispute between Pasteur and Liebig was still unsettled. However, a decision was rapidly approaching. In 1921 Copping reported that wild yeasts would grow in a solution of minerals and sugar while cultivated yeasts required the addition of bios for normal growth, and in 1924 Lash Miller and Lucas of Toronto showed that there was a difference between races of yeast in their response to bios. It seems reasonable now to suggest that the conflict in the results obtained 60 years before by Pasteur and Liebig may have been the result of differences in the strains of yeast they used.

However, although the burden of evidence seemed tipping the scales in favor of the reality of bios its chemical nature remained unknown. From 1919-1928 various unsuccessful attempts were made to identify bios with the antiberi beri vitamin of Eijkman and with the coenzyme of Harden and Young and to isolate it in crystalline form. However, Fulmer in 1923 demonstrated that bios was not a single substance, and Lash-Miller's laboratory in Toronto separated it into two fractions, Bios I and Bios II. In 1928 Eastcott showed that bios I was mesoinositol, a substance which more than ten years later was found to be necessary in the diet of chicks and of rats as well as in that of yeasts. R. J. Williams and associates separated a bios fraction which was demonstrated to be thiamine and later a fraction which proved to be a new vitamin, pantothenic acid; but a portion of bios still remained unidentified.

Kögl in Utrecht began his work in 1932 and devoted his attention to that part of the bios complex which was adsorbed on charcoal and which he called biotin. Four years later he announced the isolation of crystalline biotin. He had obtained 1.1 milligrams of the crystalline material from 250 kilograms of dried egg yolk and estimated that this amount of material originally contained a total of 80 milligrams. On this basis it would take more than 125,000 tons of dry egg yolk to yield 1 pound of biotin or, to put it another way, about 1,500,000 hens would have to work for a full year to produce the eggs necessary to yield 1 pound of pure biotin.

But this does not end the story of biotin. About 1933 it was reported that rats fed a diet high in raw egg white developed a peculiar and impressive skin injury which was accompanied by emaciation and eventually terminated fatally. This was called egg white injury. Cooked egg white did not have this effect. It was found further that egg white injury could be cured by injections of liver extract, and it was suggested that this was because of the presence in the liver extract of a new vitamin which was labelled, vitamin H. In the meantime a group of investigators in the United States Department of Agriculture had become interested in a factor which caused increased growth of the bacteria which produce nitrogen-fixing nodules on legumes. They named this factor coenzyme R. In 1940 György, Melville, Burk and du Vigneaud proved that biotin, coenzyme R and vitamin H were identical.

In the same year R. J. Williams and his associates isolated from uncooked egg white a peculiar protein, which they named avidin. Avidin it was found combines with biotin so strongly that it renders the vitamin unavailable to the organism. Egg white injury is, therefore, the result of a vitamin deficiency, a deficiency of biotin and now—biotin is suspected of having an intimate relation to cancer.

Effective quantities of the vitamins. I have spoken from time to time of effective quantities of thiamine, pyridoxine or biotin in terms of 0.01, 0.001 or even 0.0001 of a microgram, and a microgram is one millionth of a gram. This quantity of material cannot be seen, even with the most powerful microscope, and it cannot be weighed, even on the most sensitive balance. It is invisible and imponderable. If I had two dishes before me, one containing 0.001 microgram of biotin, and the other empty you could see nothing in either dish. Yet a little water rinsed in one dish and added to the proper medium would enable the proper fungus to grow while wash water from the other dish would be of no benefit.

To the uninitiated such results border on magic, and such small quantities are meaningless. What is 0.001 of a microgram? I will try to tell you. A teaspoonful of biotin weighs about 3 grams. Take one third of it and in your imagination divide it into 1000 parts. Each part would be a milligram. Take one milligram and divide it into 1000 parts. One of these is a microgram. It is only necessary to think of one microgram divided into 1000 parts to obtain 0.001 of a microgram or one trillionth of a gram. Easy to do isn't it, in your mind's eye?

But if such a small amount cannot be seen or weighed how can it be measured—anywhere else, that is, than in one's imagination. This is a simple laboratory procedure, based on the principle of dilution. If we dissolve 1 gram of biotin in a liter of pure water it is clear that one milliliter of the solution will contain 1 milligram of biotin. A milliliter can be readily and accurately measured by means of a suitable pipette. If we transfer a milliliter of solution containing a milligram of biotin to another flask of a liter of pure water and distribute it there, then one milliliter in the second flask will contain one thousandth part of a milligram, or one microgram. A third transfer of this sort will yield a solution containing per milliliter 0.001 microgram or one trillionth of a gram. To obtain such small quantities is easy, if one knows how.

How vitamins work. Such small quantities of the vitamins are effective in determining the growth of an organism, like a fungus, in comparison with the amount of some other food, such as sugar or nitrogen, that our curiosity as to how vitamins function is sure to be aroused. It appears that they are parts of enzyme systems, and enzymes are those substances found in the body which make possible the chemical changes continuously occurring in a living organism and synonymous with life itself. Much as a bit of oil speeds a huge machine, an enzyme makes chemical reactions go on which otherwise would take place very slowly indeed. Sugar dissolved in sterile water will remain unchanged indefinitely but in the presence of the proper enzyme it is broken into its parts and yields its products. Most enzymes, perhaps all, are made up of two parts, an enzyme protein and a coenzyme, neither of which is effective by itself.

Some of the vitamins are known to be precursors of coenzymes. A deficiency of one of these vitamins interferes with the activity of an enzyme system and prevents the normal metabolic changes accomplished through the agency of that system. For example, cocarboxylase is the pyrophosphate of thiamine. The enzyme, carboxylase, catalyzes the decarboxylation of pyruvic acid, one of the intermediates in the metabolism of glucose; but carboxylase is only effective in the presence of its coenzyme, cocarboxylase. When thiamine is deficient and cocarboxylase is not formed, carboxylase does not function; and the normal utilization of sugar does not occur.

How specific are the vitamins? Vitamins are highly specific; that is, nearly related compounds will not substitute for a particular vitamin. A small change in the molecular structure of a vitamin reduces its effectiveness, may eliminate its activity entirely or even change it into a harmful compound. These results are probably because of their function as coenzymes.

What vitamins are important for plants? A dozen or more chemically pure vitamins and similar substances are now available. Not all of these have been demonstrated to be important for plants because usually a plant must be discovered which is deficient for a vitamin before the need for it can be clearly demonstrated. Nevertheless, deficiencies have been found for pantothenic acid and para amino benzoic acid, the anti gray hair factors, for riboflavin, m-inositol, thiamine, biotin, pyridoxine and ascorbic acid. In the development of any plant all these vitamins are probably essential, and others too, some of which are still unidentified. Most plants, including all green plants and many of the bacteria, yeasts and fungi, construct from sugar, minerals and a source of nitrogen all the vitamins they require in amounts adequate for normal and perhaps maximum development. Furnishing these plants with vitamins does not improve their growth.

Others suffer from one or more vitamin deficiencies: that is, they do not develop satisfactorily in a medium which lacks vitamins. Some plants have a *complete* deficiency for one or more vitamins. They are unable to synthesize any of the vitamin (or vitamins) in question, and in its absence do not grow. This is true of *Phycomyces* for thiamine. Others suffer from *partial* deficiencies; that is, they grow slowly in the absence of the particular vitamin, but more rapidly if it is present in the medium. Apparently they are able to make some of the vitamin, but not enough for maximum growth. Both complete and partial deficiencies may be *single* (for one vitamin) or *multiple* (for more than one vitamin). The deficiency may be *absolute,* or it may be *conditioned.* By an absolute deficiency I mean that no known environmental conditions enable the organism to synthesize the vitamin from the simple foods and nutrients in a vitamin-free medium. This appears to be true of *Phycomyces* in its relation to thiamine. *Pythium butleri,* on the other hand, suffers from a thiamine deficiency in a concentrated mineral solution which is relieved by diluting the solution. Its deficiency is conditioned by the medium in which it is grown.

The synthetic ability of a plant for a particular vitamin may be *complete, incomplete, or none;* that is, some plants are able to construct the vitamin from simple food and nutrients; others are capable of making the vitamin if supplied one or all of its intermediates; and still others are incapable of constructing any portion of the vitamin. For example, *Aspergillus niger* has complete synthetic power for thiamine; it can make this substance if supplied with sugar and minerals including nitrates. On the other hand, *Phytophthora cinnamomi* must be supplied with thiamine as such. It apparently lacks the ability to synthesize any portion of the thiamine molecule, resembling the animal in this respect. Between the two extremes of no synthetic power and complete synthetic ability, there exist many types of incomplete synthetic power. For example, *Mucor ramannianus* can make the pyrimidine half of the thiamine molecule but not the thiazole portion; *Sclerotium rolfsii* can make the thiazole but not the pyrimidine part; *Phycomyces* can combine the two intermediates into the thiamine molecule but is incapable of making either.

The importance of studies on vitamins in relation to plants. By this time you may be willing to admit that plants need vitamins, but you may still question the importance of such knowledge, particularly when you remember what I have said on the negative results of applying vitamins to green plants in field or garden. If most plants make all the vitamins they need, why should we study the subject?

There are many reasons. Even though vitamin B_1 seems at present to exist in sufficient amounts in green plants to permit them to develop satisfactorily without giving them more of it, there are many other vitamins, some as yet unidentified, and we know relatively little of their relations to the growth of green plants. If we search further, we may *perhaps* find a vitamin not made by green plants in large enough quantity for their maximum development—one which when fed to the plant will actually perform part of the miracles our commercially-minded friends have told us could be accomplished with vitamin B_1; but, at present, this seems like a rather long chance.

However, it is quite necessary for us to understand the nutrition of bacteria, yeasts and molds, a good many of which suffer from vitamin deficiencies. These lower plants are most important in causing disease and decay as well as bringing benefits through their relation to fermentation, cheese making and many similar processes. If we wish to control these lower plants—limit their detrimental activities and encourage their beneficial properties—we must understand how they live. For example, the edible morel is one of the most delicious of fungi, far superior to the mushroom we buy in the markets. Yet no one has ever cultivated it. Why? Perhaps it suffers from vitamin deficiencies which have never been properly satisfied by the materials upon which men have tried to grow it.

But even if further research should show that the use of vitamins on plants is of no practical significance, the study of the relation of vitamins to plants is important because of the light it may throw on their uses for animals. Plants have been found to be valuable tools in determining the presence and in estimating the quantity of various vitamins, in indicating how vitamins work in the animal and in leading investigators to the discovery of new vitamins. Pantothenic acid, paraamino-benzoic acid, inositol and biotin were all discovered through their effects on plants before they were known to have any influence on animals.

Entirely aside from the practical importance of using plants to increase our knowledge of a class of substances so important for animals, or of using the substances themselves to influence and modify the development of plants, it should help to reëstablish self-respect in the human race at the present moment in world history, to learn that in certain fundamental ways we resemble the innocent and harmless yeast plant. The same vitamins are concerned in the development of yeast as in the growth of man and they probably perform the same functions in both organisms.

And so science weaves a magic carpet of Bagdad which can carry us over the mountains and through the jungles which once impeded and entangled the footsteps of the seeker for knowledge. Threads from far off China, a bit of material from Java, some from all the world are woven in its woof. There are times when it is necessary to unravel a bit of the weaving unsuited to the pattern, but in the end the carpet is woven, and with its aid you can scale heights which neither Liebig nor Pasteur could surmount.

THE NEW YORK BOTANICAL GARDEN
NEW YORK, NEW YORK